

# Oat and Rye Root Decomposition Effects on Nitrogen Mineralization

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## ABSTRACT

Decomposition and mineralization of cover crop roots needs to be understood to determine if N taken up by cover crops is mineralized during main crop growth. Two experiments were conducted in a controlled environment to measure decomposition of oat (*Avena sativa* L. 'Ogle') and rye (*Secale cereale* L. 'Rymin') root residues and to examine its effect on soil N mineralization. In the first, oat and rye roots were mixed with soil and in the second, roots were grown in situ. At 7, 14, 28, 56, 84, and 112 d after the start of decomposition, denitrification, soil  $\text{NO}_3^-$ , and soil  $\text{NH}_4^+$  were measured to determine net mineralized N. Soil respiration and C and N contained in roots and coarse soil organic matter were measured to determine decomposition. All treatments in both experiments showed an increase in net mineralized N during the first 56 d. After 56 d, net mineralized N in the control remained relatively constant, whereas mineral N continued to accumulate in the treatments with root residues. Net N mineralization of the rye and oat root treatments did not differ. Roots mixed with soil had high respiration rates during the first 3 d and there were no differences between oat and rye root treatments. In the roots in situ experiment, however, respiration peaked for oat roots at Day 12 and for rye roots at Day 33. The oat treatment also had less C and N remaining in roots and coarse organic matter throughout the experiment. Even though oat roots decomposed faster than the rye roots, we predict that <55% of the N contained in the roots of a spring-killed oat or rye cover crop will become available to the following crop.

CURRENT AGRICULTURAL PRACTICES for row crops, such as corn (*Zea mays* L.), have resulted in increased  $\text{NO}_3^-$  concentrations in surface and ground water sources of drinking water (Baker et al., 1975; Meisinger et al., 1991; Brandi-Dohrn et al., 1997). The use of winter cover crops is a management practice, which can reduce  $\text{NO}_3^-$  leaching and subsequent contamination of water supplies (Meisinger et al., 1991; Reicosky and Warnes, 1991; Herbert et al., 1995; McCracken et al., 1995; Brandi-Dohrn et al., 1997). Cover crops reduce nitrate leaching between cropping seasons because they take up  $\text{NO}_3^-$  during growth and because  $\text{NO}_3^-$  is immobilized when their residues decompose.

Normally, nonleguminous residues cause N immobilization during the first few weeks of decomposition due to their high C/N ratio (Doran and Smith, 1991; Somda et al., 1991; Green and Blackmer, 1995). For example, Green et al. (1995) observed that corn stover added to the soil resulted in rapid immobilization of all available inorganic N during the period of rapid decomposition. If rapid decomposition of cover crop residues occurs during main crop growth, then the availability of  $\text{NO}_3^-$  for the main crop may be reduced. Eventually, as

decomposition proceeds, the C/N ratio of the residues approach that of soil organic matter, the microbial biomass decreases, and N from the residues or soil that was incorporated into the microbial biomass is released into the soil. If residue decomposition proceeds too slowly, N may not be released from the residues and microbial biomass before the main crop has matured and has stopped taking up N. For example, Varvel and Peterson (1990) hypothesized that in a continuous corn system, 80% of the applied fertilizer N was still immobilized in crop residues, soil organic matter, and microbial biomass at the end of the growing season. Thus, understanding the factors that control cover crop decomposition is important for reducing  $\text{NO}_3^-$  leaching losses and synchronizing  $\text{NO}_3^-$  availability with main crop uptake.

Information on decomposition of residue derived from nonleguminous cover crops is limited and often incomplete (Ditsch and Alley, 1988). Even though there is some information about the general pattern of shoot residue decomposition, less is known about root residue decomposition. Xu and Juma (1995) found that 39 and 57% of the in situ root residues from two varieties of barley (*Hordeum vulgare* L.) were respired in 10 d after removing the shoots. Broadbent and Nakashima (1974) reported that 88 and 85% of C was lost from barley tops and roots, respectively, after 5 yr, whereas 56 and 41% of N was mineralized during the same period of time. Additional, information on decomposition of cover crop root residues and the differences among cover crop species is needed.

Residue disturbance or incorporation caused by tillage influences residue decomposition rates. In general, incorporation of shoot residues by tillage results in faster residue decomposition. Root residues, however, may respond differently to tillage or disturbance. Martin (1989) observed that root residues decomposed more rapidly and completely when roots were left undisturbed in the soil than when air-dried roots were mixed with moist or air-dried soil. Martin hypothesized that undisturbed roots decomposed more rapidly because the roots had established intimate contact with soil particles and microbial colonization of the root surface had already occurred. In contrast, in disturbed or air-dry soil root decomposition was delayed because the root surface had to be recolonized by microbes before decomposition could begin.

An understanding of decomposition rates of cover crop root residues and their effects on net soil N mineralization is needed to determine if N taken up by cover crops is mineralized during main crop growth. This will allow the development of cover crop management systems that reduce  $\text{NO}_3^-$  leaching and improve N-use efficiency. As part of a larger research project that is exam-

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ining the use of oat and rye as winter cover crops following soybean [*Glycine max* (L.) Merr.] in the upper Midwest (Johnson et al., 1998), we conducted two controlled environment experiments. In the first, oat and rye roots were mixed with soil and in the second, roots were grown in situ. The objectives of these experiments were to determine and compare decomposition rates of oat and rye root residues mixed with soil or grown in situ, and to examine the effect of oat and rye root residues on net soil N mineralization.

## MATERIALS AND METHODS

This study was conducted in the greenhouse and growth chambers of the National Soil Tilth Laboratory in Ames, IA, and consisted of two experiments. The experiments measured decomposition of oat and rye roots and net mineralized N in soil under controlled conditions over 112 d. In Exp. 1, fresh oat and rye root residues were mixed with dry soil. In Exp. 2, oat and rye roots were grown in situ.

Experiment 1, oat and rye roots mixed with soil, had a completely randomized design with five replications, five root residue treatments, and six sampling dates. Root residue treatments consisted of 0.30 and 0.60 g of fresh oat roots, 0.30 and 0.60 g of fresh rye roots, and no roots for the control. Experimental units were destructively sampled at 7, 14, 28, 56, 84, and 112 d after the roots were mixed with the soil. The total number of experimental units was 150. Approximately 20 kg of a Canisteo [fine-loamy, mixed (calcareous), mesic Typic Endoaquolls] soil was collected from the Iowa State University Agronomy and Agricultural Engineering Research Center located about 11 km west of Ames, IA. The soil was air dried, mixed, passed through 5 and 2 mm sieves, and mixed again. Soil and organic matter remaining on top of the sieves was discarded. Each experimental unit consisted of a 150 g of air-dry, sieved soil contained in a polycarbonate tube (30 cm long  $\times$  4 cm diam.). For experimental units that contained oat or rye roots, roots were obtained by washing the roots from 42-d-old plants grown in sand in the greenhouse. Fresh roots were then cut into pieces 10 to 20 mm long. To mix the measured weight of roots and the soil for each oat and rye root experimental unit, the 150 g of soil was spread out on a flat surface, the roots were evenly distributed on top of the soil, and then mixed. The soil and root mixture was then poured carefully into each tube to prevent layering of roots or fine soil particles. Control treatment experimental units had 150 g of soil, but no roots. The bulk density of the soil in the tubes was approximately  $1.02 \text{ g cm}^{-3}$  and was adjusted if necessary by tapping the tubes to settle the soil until it occupied a standard volume. After the soil and root mixture had been poured in, the tubes were weighed, wetted to 50% water-filled pore space ( $\approx 43 \text{ mL}$  of water at bulk density of  $1.02 \text{ g cm}^{-3}$ ), weighed again, and then rewetted every 15 d to the same water content as determined by weight. The tubes were capped with a rubber stopper at the lower end. The upper ends of the tubes were capped with foam plugs to allow aeration, but to reduce evaporation. All tubes were kept in a growth chamber at  $25^\circ\text{C}$ , 80% humidity, and no light.

Experiment 2, oat and rye roots grown in situ, had a completely randomized design, six replications, three root residue treatments, and seven sampling dates. The three root residue treatments were oat roots grown in situ, rye roots grown in situ, and no roots. The sampling dates were 0, 7, 14, 28, 56, 84, and 112 d after the start of the experiment. The number of replications was 6, so the total number of tubes was 126. Oat and rye seeds ( $n = 200$ ) were weighed to determine the

mean seed weight and standard deviation. Using a weight criteria of the mean  $\pm 0.5$  standard deviation, 42 seeds of each species were selected for the experiment. The seeds were pregerminated on germination paper and then one seed was placed on the surface of 150 g of air-dry Canisteo soil in each polycarbonate tube (same size of tubes as Exp. 1). The soil was taken from the same location as that used in Exp. 1, but it was collected in the fall instead of early summer. The soil in the tubes was wetted to 50% water-filled pore space and then capped with foam plugs. The soil was rewetted to the same water content every 15 d. The plants were grown for 42 d in a growth chamber at  $25^\circ\text{C}$ , 80% humidity, and 10 h light. After 42 d, shoots were cut off at the soil surface and removed. After the shoots were removed, the growth chamber was set at  $25^\circ\text{C}$ , 80% humidity, and no light for 112 d. As before, the soil was rewetted to the same water content every 15 d. Shoots were dried at  $55^\circ\text{C}$  and weighed to  $\pm 0.0001 \text{ g}$ .

Changes in net N mineralized during the 112 d incubation were calculated from the increases in soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , and from denitrification losses. Denitrification rates were measured using the acetylene inhibition method (Parkin, 1987). Vials containing 8 mL of air taken from the tubes were analyzed for nitrous oxide ( $\text{N}_2\text{O}$ ) using a gas chromatograph equipped with  $^{63}\text{Ni}$  electron capture detector which is operated at  $300^\circ\text{C}$ . After denitrification rates were determined, soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in the entire 150 g of soil were extracted with 600 mL of a 2 M KCl solution (Hart et al., 1994). Jars containing the soil and KCl solution were shaken for an hour and then allowed to settle for another hour before a 10-mL sample was taken. The sample was filtered with a Whatman<sup>1</sup> No. 41 filter (Whatman Int. Ltd. Maidstone, England). Filtered extracts were analyzed using colorimetric determination of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (Bundy and Meisinger, 1994). Net N mineralized was calculated by adding  $\text{NO}_3^-$  and  $\text{NH}_4^+$  content of soil to cumulative denitrification-N loss. Data are presented as  $\mu\text{g}$  of N per g of soil and  $\text{NO}_3^-$  and  $\text{NH}_4^+$  contents of soil at Day 0 are included in the total.

Root and coarse organic matter decomposition, and C and N contents were monitored by recovering roots and coarse organic matter from the entire 150 g of soil in each tube. After the KCl extraction and mineral N sampling was completed, sodium hexametaphosphate was added to the soil and KCl solution to disperse soil aggregates. After 48 h, the soil and KCl solution was sieved through a 500- $\mu\text{m}$  screen. Sand particles larger than 500  $\mu\text{m}$  were separated from coarse organic matter and roots by repeated agitation in water and decanting. Thus, for the purposes of this experiment we define coarse organic matter as being larger than 500  $\mu\text{m}$ . The coarse organic matter and roots were dried at  $55^\circ\text{C}$  and weighed to  $\pm 0.0001 \text{ g}$ . The dried coarse organic matter and roots (also shoots from Exp. 2) were then ground and analyzed for total C and N using the dry combustion method with a Carlo-Erba NA 1500 NCS elemental analyzer (Haake Buchler Instruments, Paterson, NJ). Data are presented as  $\mu\text{g}$  of C or N contained in roots and coarse organic matter per g of soil.

Soil respiration was measured two or three times a week to monitor decomposition. At each measurement time, 25 tubes of Exp. 1 (roots mixed with soil) and 18 tubes of Exp. 2 (roots in situ) were measured. Carbon dioxide concentration was measured with a CI-301  $\text{CO}_2$  infrared gas analyzer (CID, Inc., Vancouver, WA). A rubber stopper fitted with two lengths of plastic tubing connected to the infrared gas

<sup>1</sup> Reference to a trade or company name is for specific information only and does not imply approval or recommendation of the company or product by the USDA to the exclusion of others that may be suitable.

analyzer was placed on each tube, in turn. Headspace gas in the tube was then recirculated through the gas analyzer in a closed loop and the  $\text{CO}_2$  concentrations were determined every 12 s for 300 s. Respiration rates were determined by calculating the slope of the linear regression of  $\text{CO}_2$  concentrations and time.

Data from each experiment and sampling date were analyzed separately. Analysis of variance was used to detect differences among treatment means. An LSD test at 0.05 probability level was used to compare treatment means when the analysis of variance indicated significant treatment effects at 0.05 probability level (Cochran and Cox, 1992).

## RESULTS AND DISCUSSION

Soil  $\text{NO}_3^-$  was the largest component of our estimates of net mineralized N, in both experiments. Soil ammonium content, although less than soil nitrate, was a significant component of the mineral N, especially early in both experiments. The denitrification rate had minimal effects on soil N levels or estimates of net N mineralized. In both experiments, total denitrification-N losses were  $<0.5 \mu\text{g N g soil}^{-1}$ . Aulakh et al. (1991) found that soil denitrification was negligible at 60% water-filled pore space, which is consistent with the results of this experiment at 50% water-filled pore space.

All treatments in Exp. 1, roots mixed with soil, showed an increase in net N mineralized during the first 56 d (Fig. 1). After 56 d, the control's N levels decreased, whereas N concentrations continued to increase slightly in the treatments with root residues. Nitrogen mineralization did not differ significantly among the oat and rye root treatments. At the end of the experiment, however, net N mineralized from the control was statistically lower than any of the root treatments. At Day 112, net N mineralization in the control was  $14.8 \mu\text{g N g soil}^{-1}$  (46% increase from Day 0), and N mineralization in the oat and rye root treatments averaged  $23.2 \mu\text{g N g soil}^{-1}$  (131% increase from Day 0) and  $21.7 \mu\text{g N g soil}^{-1}$  (116% increase from Day 0), respectively. In the root treatments more than 30% of the increase in the net N mineralized occurred in the first 7 d. It is interesting to note that net immobilization of N was not observed. Often, during the initial decomposition of organic matter with a high C/N ratio N is immobilized (Green and Blackmer, 1995). Nitrogen mineralized during the first 56 d after rewetting was probably partly due to the decomposition and/or release of N from organic matter and microbial biomass that was present in the soil during drying. It is not uncommon to observe a burst of N mineralization soon after soil is rewetted from an air-dry condition (Black, 1968; Murphy et al., 1998). Drying increases the susceptibility of some of the soil organic matter to decomposition through physical disruption, causes turnover of microbial biomass, and may cause the release of bound ammonium (Black, 1968).

Generally, the N contained in the roots and the coarse organic matter in Exp. 1 decreased gradually through Day 84 (Fig. 2). This is the inverse of the general pattern for net N mineralized (Fig. 1). The control and the root treatments did not differ and lost between 40 and 54% of the N contained in the roots and coarse organic matter.

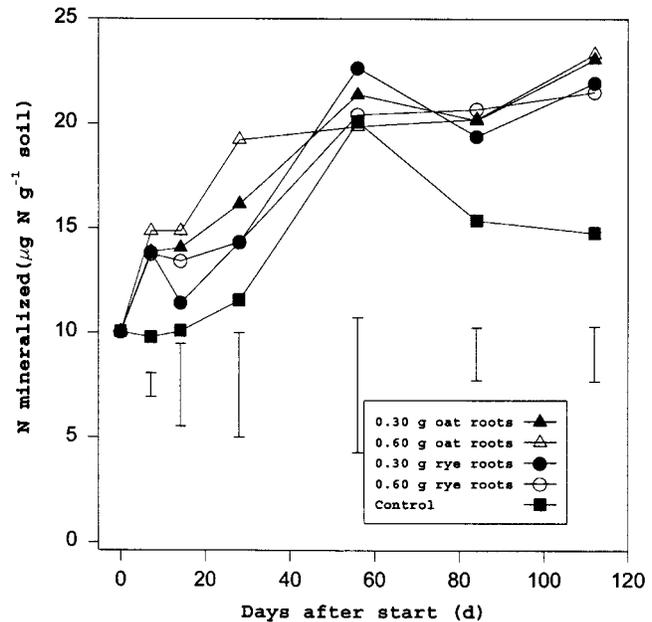


Fig. 1. Net N mineralized (includes soil  $\text{NO}_3^-$  + soil  $\text{NH}_4^+$  + N lost through denitrification) during Exp. 1 (roots mixed with soil) plus initial soil N content. Bars represent LSD(0.05).

Nitrogen mineralized during these incubations cannot be accounted for entirely by changes in the root/coarse organic matter fraction. Over the time interval of Days 7 to 112, net N mineralized in the oat and rye treatments was  $8.9$  to  $7.9 \mu\text{g N g soil}^{-1}$ , respectively (Fig. 1). Over this same time interval  $3.3$  and  $3.4 \mu\text{g N g soil}^{-1}$  was lost from the root and coarse organic matter fraction of these treatments (averaged from Table 1). The extra N observed in the soil mineral N pool ( $5.6 \mu\text{g N g soil}^{-1}$  for the oat and  $4.5 \mu\text{g N g soil}^{-1}$  for the rye) must have come from the unmeasured pools of organic N in the soil, such as soluble organic N, particulate organic matter  $< 500 \mu\text{m}$ , or microbial biomass. The control treatment showed a similar result, in that more N accumulated in the mineral N pool from Days 7 to Day 112 ( $5.0 \mu\text{g N g soil}^{-1}$ ) than could be accounted for by decreases in the N pool of coarse organic matter  $> 500 \mu\text{m}$  ( $1.9 \mu\text{g N g soil}^{-1}$ ).

In Exp. 2, roots in situ, the control had higher levels of net N mineralized than those of oat and rye root treatments through Day 84 (Fig. 3). The difference in starting N concentrations can be accounted for by N uptake by the oat and rye plants during the 42 d before the decomposition experiment started. During this 42 d period the soil in the control, which did not have plants, was kept moist and accumulated N through mineralization. The N taken up by the oat and rye shoots nearly matched the starting inorganic N level in the control ( $21.2 \mu\text{g N g}^{-1} \text{ soil}$ ). Subtracting the N contained in the seed (equivalent to  $4 \mu\text{g N g}^{-1} \text{ soil}$ ) at planting, the oat shoots removed from the soil on average  $24 \mu\text{g N g}^{-1} \text{ soil}$ , and the rye shoots took up the equivalent of  $17 \mu\text{g N g}^{-1} \text{ soil}$ . This apparent balance between uptake and available inorganic N, however, does not consider the N taken up by the plant and contained in the roots. We did not separate roots from coarse organic matter

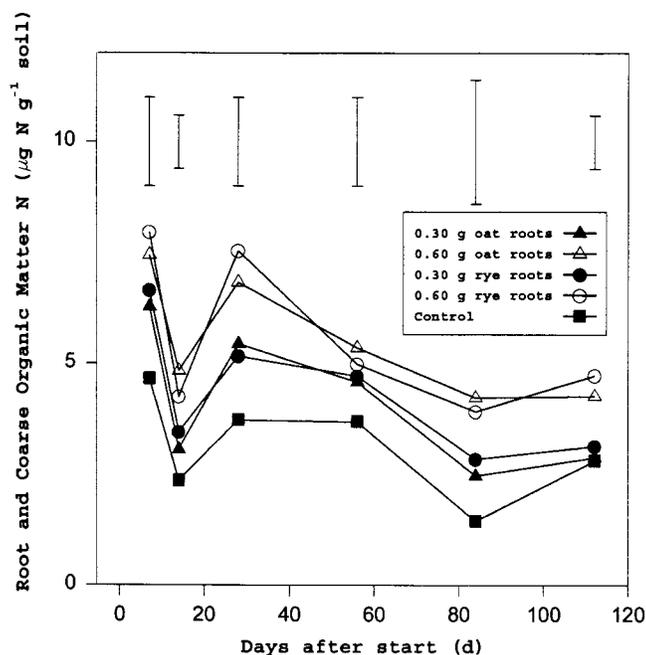


Fig. 2. Nitrogen contained in roots and coarse organic matter during Exp. 1 (roots mixed with soil). Bars represent LSD(0.05).

in the soil and thus, we do not have a direct measure of root N. An estimate of root N can be calculated, however, by subtracting the N contained in the coarse organic matter of the control treatment (no roots) from the N contained in the coarse organic matter and roots of the oat and rye treatments (Table 1). Using this estimation the oat roots contained  $3.3 \mu\text{g N g}^{-1}$  soil and the entire oat plant  $27.3 \mu\text{g N g}^{-1}$  soil. The rye roots contained  $10.2 \mu\text{g N g}^{-1}$  soil and the entire rye plant  $27.2 \mu\text{g N g}^{-1}$  soil. These values are remarkably close to each other and seem to be larger than the inorganic N level in the control ( $21.2 \mu\text{g N g}^{-1}$  soil) on Day 0. We are unsure of the significance of these estimations, but other studies have reported a stimulatory effect of plants on net mineralization (Haider et al., 1989; Wheatley et al., 1990).

After shoots were removed on Day 0 (Fig. 3), rates of net N mineralization were nearly the same for the three treatments through Day 56 ( $0.206$ ,  $0.204$ , and  $0.223 \mu\text{g N g}^{-1}$  soil  $\text{d}^{-1}$  for the control, oat, and rye treatments, respectively). After 56 d the N mineralization rate of the control decreased from  $0.206$  to  $0.020 \mu\text{g N g}^{-1}$  soil  $\text{d}^{-1}$  and the soil N level remained relatively constant. In contrast, the soil N concentrations of the oat and rye treatments continued to increase at about the same rate as before Day 56. We speculate that most of the readily-decomposable organic matter in the control had been decomposed by Day 56. On the other hand, the soil with the oat and rye root residues still had readily decomposable organic matter available after Day 56 and mineralization continued. By Day 112 the levels of net N mineralized in the oat and rye root treatments were no longer significantly less than that of the control (Fig. 3). As in our experiment, Hoyt and Mikkelsen (1991) also observed that nonlegume cover crops accumulate N during their growth and then recycle

Table 1. Nitrogen contained in roots and coarse organic matter† at Days 0 and 112 in two experiments.

Experiment	Treatment	Time			% loss
		Day 0‡	Day 112	Difference	
		$\mu\text{g N g}^{-1}$ soil			
Exp. 1	Control	4.7b§	2.8c	1.9a	40
Roots mixed with soil	0.30 g Oat	6.0ab	2.9bc	3.4a	54
	0.60 g Oat	7.3a	4.3ab	3.2a	43
	0.30 g Rye	6.7a	3.2bc	3.5a	52
	0.60 g Rye	8.0a	4.7a	3.3a	41
Exp. 2	Control	11.5b	11.8a	-0.3a	0
Roots in situ	Oat	14.8b	11.2a	3.6a	24
	Rye	21.7a	12.4a	9.3a	43

† Coarse organic matter is the organic matter remaining on a  $500\text{-}\mu\text{m}$  sieve after repeated agitation and washing.

‡ In Exp. 1 the first measurement was made on Day 7.

§ Means with the same letter within an experiment and column are not different at the 0.05 probability level, as indicated by the LSD test.

the N taken up during the decomposition of cover crop residues.

The N contained in the roots and coarse organic matter in Exp. 2, roots in situ, decreased during the first 28 d, and then remained relatively constant (Fig. 4). On the other hand, net N mineralized continued to increase steadily throughout the experiment for the oat and rye root treatments (Fig. 3). Nitrogen losses from the root and coarse organic matter fraction are calculated by changes in this pool from Days 0 to 112 (Table 1). The oat root treatment lost 24% of the N contained in the roots and coarse organic matter over 112 d, whereas the rye root treatment lost 43%. The control did not lose a measurable amount of N from the coarse organic matter. The N losses from the root and coarse organic matter fraction do not account for the increases in mineral N noted in Fig. 3. Like Exp. 1, it is likely that net N mineralization was supported by N losses from other pools such as the soluble fraction, microbial biomass,

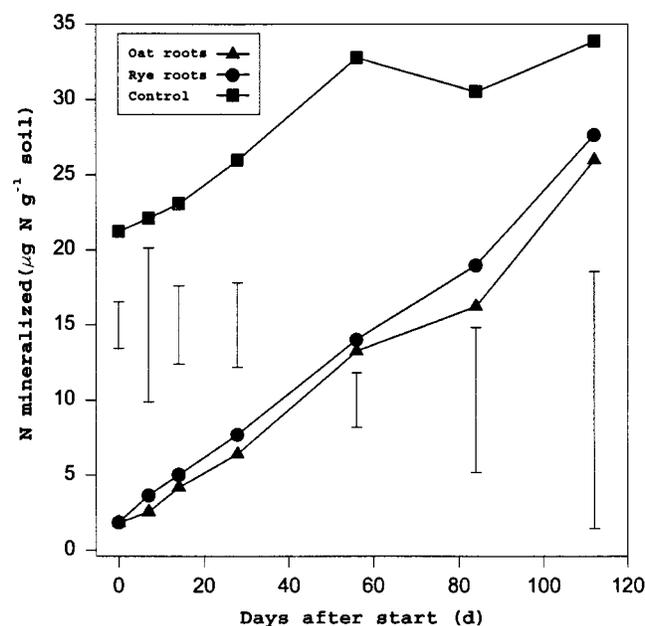


Fig. 3. Net N mineralized (includes soil  $\text{NO}_3^-$  + soil  $\text{NH}_4^+$  + N lost through denitrification) during Exp. 2 (roots in situ) plus initial soil N content. Bars represent LSD(0.05).

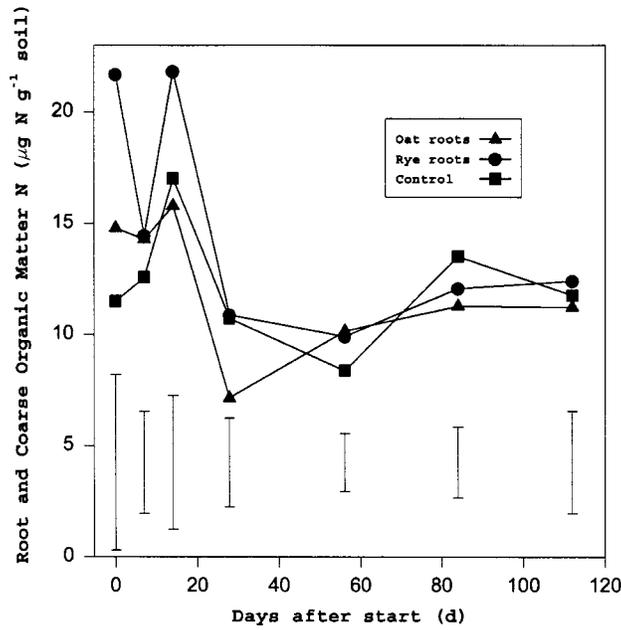


Fig. 4. Nitrogen contained in roots and coarse organic matter during Exp. 2 (roots in situ). Bars represent LSD(0.05).

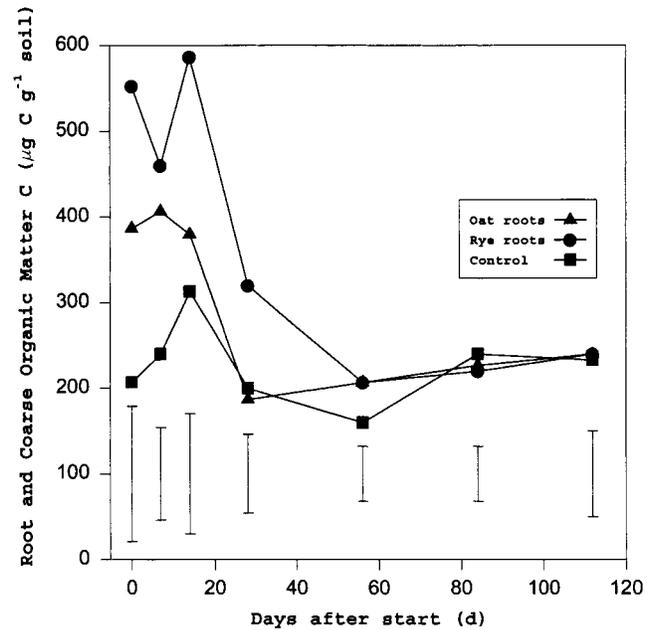


Fig. 6. Carbon contained in roots and coarse organic matter during Exp. 2 (roots in situ). Bars represent LSD(0.05).

or particulate organic matter < 500 µm in size. We calculated by subtraction that these other sources contributed 21.5, 17.4, and 13.4 µg N g soil<sup>-1</sup>, to the net N mineralized in the oat, rye, and control treatments, respectively.

The C contained in the oat and rye root residues and coarse organic matter in both experiments decreased more rapidly during the first 56 d than from 56 to 112 d (Fig. 5 and 6). In Exp. 1, roots mixed with soil, there were no additional decreases in C content after Day 84. The C contained in the control's coarse organic matter generally was significantly less than that of the oat and

rye root treatments. Oat and rye treatments were not significantly different, but 0.60-g treatments usually had significantly larger C contents than the 0.30 g treatments. The decrease in C content between 0 and 112 d was significantly greater in the treatments with roots than in the control (Table 2), but did not differ between oat and rye treatments or between different amounts of roots.

In Exp. 2, roots in situ, most of the change in C in the roots and coarse organic matter occurred in the first 28 d and only the rye treatment continued to decrease between Days 28 and 56. The oat treatment had a significantly lower C content than the rye treatment during the first 28 d probably due to less root dry matter than the rye treatment on Day 0. The oat treatment's C content was not different from the control after decomposing for 14 d, but it took 56 d before the rye root treatment had decreased to the same level as the control. At the end of the experiment, the oat root treatment showed a 38% C loss, whereas rye root treatment had a 53% loss (Table 2). The control treatment did not show a measurable loss of C from its coarse organic matter and lost significantly less organic matter than the rye root treatment.

Respiration rate measurements showed that the roots had a different decomposition pattern depending on the way they were incorporated in the soil. In Exp. 1, roots mixed with soil, all five treatments showed a peak respiration rate immediately after Day 0 (Fig. 7). This peak corresponds to an early increase in mineralized N (Fig. 1). Other studies also have shown an immediate peak in respiration rates upon addition of plant residues and water (Nowak and Nowak, 1990; Ladd et al., 1995; Xu and Juma, 1995). After approximately 59 d, respiration rates in all treatments remained low through Day 112. The control also had a slightly elevated rate during the

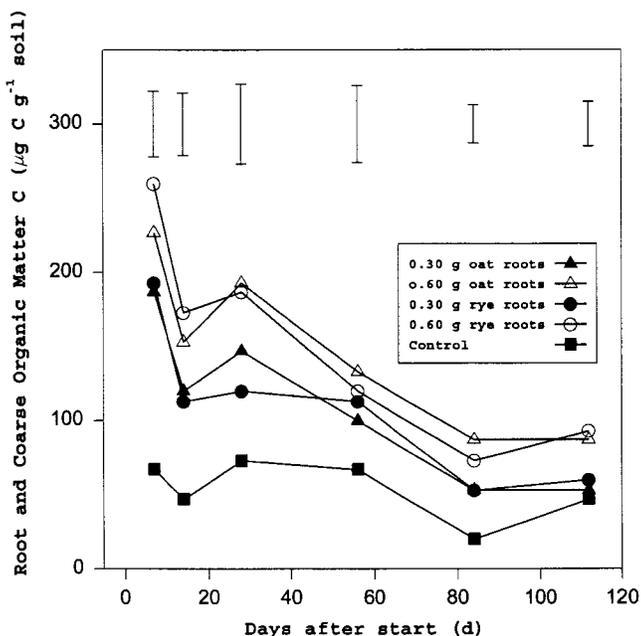


Fig. 5. Carbon contained in roots and coarse organic matter during Exp. 1 (roots mixed with soil). Bars represent LSD(0.05).

**Table 2. Carbon contained in roots and coarse organic matter† at Days 0 and 112 in two experiments.**

Experiment	Treatment	Time			
		Day 0‡	Day 112	Difference	% loss
		$\mu\text{g N g}^{-1} \text{ soil}$			
Exp. 1	Control	67c§	47a	20b	30
Roots mixed with soil	0.30 g Oat	187b	53a	134a	72
	0.60 g Oat	227ab	87a	140a	62
	0.30 g Rye	191b	61a	130a	68
	0.60 g Rye	260a	93a	167a	64
Exp. 2	Control	207c	233a	-26b	0
Roots in situ	Oat	387b	240a	147ab	38
	Rye	553a	260a	293a	53

† Coarse organic matter is the organic matter remaining on a 500- $\mu\text{m}$  sieve after repeated agitation and washing.

‡ In Exp. 1 the first measurement was made on Day 7.

§ Means with the same letter within an experiment and column are not different at the 0.05 probability level, as indicated by the LSD test.

first 16 d, but then remained low throughout the rest of the study and usually had rates lower than those of the other treatments. This initial peak in respiration after rewetting was probably caused by the decomposition of both coarse soil organic matter and organic matter smaller than 500  $\mu\text{m}$  that had been exposed by soil drying. The treatments with roots were significantly different from the control during the first 59 d. Treatments with 0.60 g of roots had higher rates than those with 0.30 g of roots during the first 16 d. There were no significant differences between oat and rye treatments. After 59 d, none of the treatments were statistically different.

The pattern was different in Exp. 2 with roots in situ (Fig. 8). There was a lag between the beginning of the experiment and the peak respiration rates of oat and rye root treatments. Oat roots produced a peak respiration rate at approximately Day 14 and rye at Day 33. The control did not show any peak and remained relatively constant during the 112 d probably because most of the readily decomposable organic matter had already been decomposed while the soil was kept moist for 42 d prior to the start of the experiment. The average respiration rate of the control in Exp. 2 ( $2.8 \mu\text{g CO}_2\text{-C g}^{-1} \text{ soil d}^{-1}$ ), however, was equal to the peak respiration rate of the control in Exp. 1 (peak =  $2.5 \mu\text{g CO}_2\text{-C g}^{-1} \text{ soil d}^{-1}$ ; avg. =  $1.2 \mu\text{g CO}_2\text{-C g}^{-1} \text{ soil d}^{-1}$ ), indicating that the base level of microbial activity in Exp. 2 was higher than that in Exp. 1. After  $\approx 55$  d, the rates of all three treatments stayed relatively low through Day 112. The treatments were significantly different only at some dates during the first 50 d. The oat peak rate was significantly larger than the rye and control rates. Although rye rates were significantly different than the control at some dates, the rye peak rate was not significantly different from the control and oat treatment because of the large variation in respiration rates among rye replications at that time. We think that part of this variation was caused by the lack of synchronization among reps for the timing of the peak respiration event.

A possible explanation for the different pattern of root decomposition in the two experiments is that in Exp. 2, roots in situ, the roots remained alive for a period of time after shoots were removed. Possibly, the carbohydrates stored in the roots allowed them to

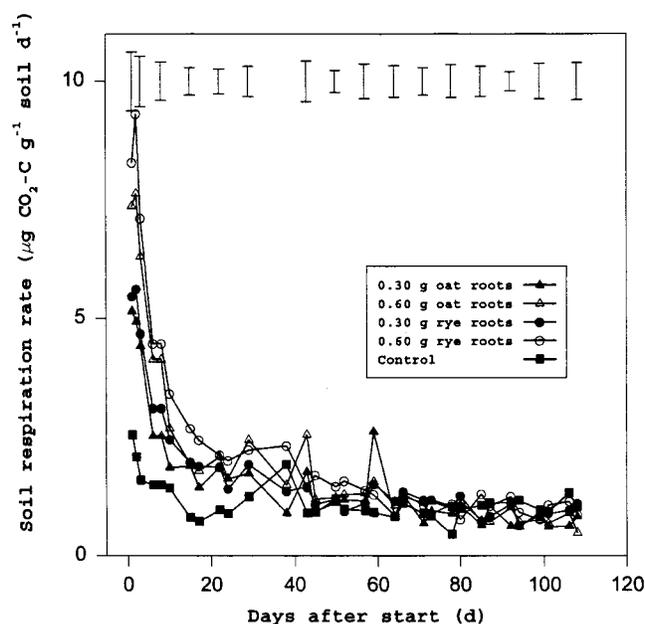


Fig. 7. Soil respiration rate during Exp. 1 (roots mixed with soil). Bars represent LSD(0.05).

survive for a while, but after the carbohydrates were exhausted, the roots died, and decomposition commenced. Conversely, we speculate that in Exp. 1, roots mixed with soil, the roots were already dead when they were added to the soil because they had been cut from the shoots several days before that. Consequently, decomposition probably began right away and produced the peak rates in the first 14 d. Another hypothesis is that microorganisms in the in situ experiment did not have enough N in the soil to begin decomposing roots immediately (Fig. 3). These hypotheses should be tested with additional research.

Another important result of Exp. 2 is that the decom-

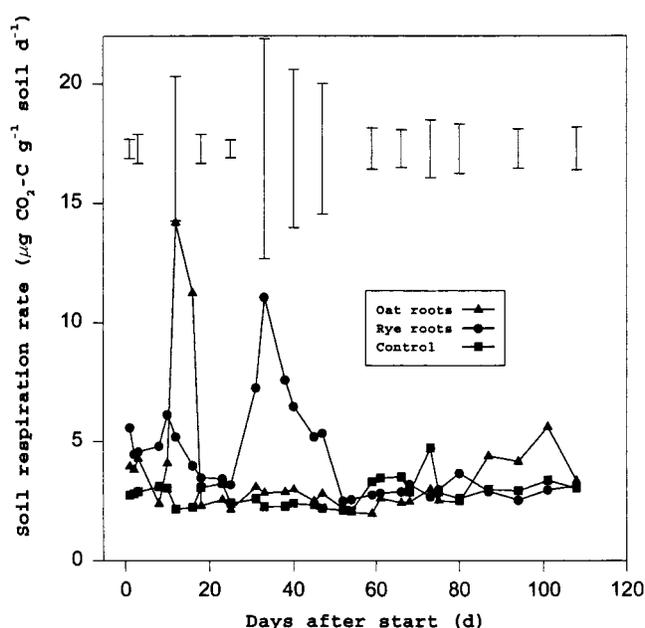


Fig. 8. Soil respiration rate during Exp. 2 (roots in situ). Bars represent LSD(0.05).

position pattern of oat roots differed from rye roots. A possible hypothesis to explain this difference is that rye roots lived longer than oat roots after removing the shoots and, therefore, the oat roots decomposed sooner than rye roots. Another reason could be that rye roots produce toxic compounds when they were still alive and these compounds killed some of the microbes present in the soil. When the roots died, the microbe populations took some time to recover and begin to decompose the roots. A third hypothesis is that oat and rye roots have different chemical composition, as Nowak and Nowak (1990) proposed for the differences in decomposition rates between roots and shoots of plants. If oat roots have more readily-decomposable compounds than rye roots, then oat roots would produce a peak respiration rate earlier than rye roots. These hypotheses should be addressed by further studies.

## CONCLUSIONS

Root inputs of plant-derived organic matter can be substantial, yet little is known about root decomposition and its subsequent effect on N mineralization. Our studies on oat and rye root decomposition revealed several important observations. First, incorporation of oat and rye roots into soil in a simulated tilled system resulted in the stimulation of net N mineralization over 112 d. The equivalent of <50% of the N contained in the root residues, however, was released. Second, there were no differences in net N mineralized between oat and rye root treatments in either experiment. Third, the pattern of oat and rye root decomposition depends on the way in which roots are incorporated into the soil. When roots were mixed with the soil, the initial high respiration rates and the rapid decrease in C contained in roots and coarse organic matter showed that the roots began decomposing right away. Alternately, when roots were grown in situ, there was a lag before the peak respiration rates occurred. This lag may have occurred because the roots did not die immediately after the shoots were cut off and because the soil was almost N depleted. Lastly, the decomposition pattern was affected by the amount and type of roots. In the roots mixed with soil study, differences in C content and respiration rates were detected when different amounts of roots were added to the soil. In the study with roots in situ, oat roots decomposed more rapidly than rye roots. We cannot completely explain these observations and further studies are needed.

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## Arylamidase Activity of Soils

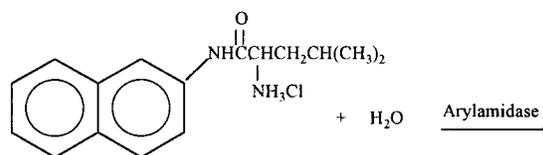
V. Acosta-Martínez and M. A. Tabatabai\*

### ABSTRACT

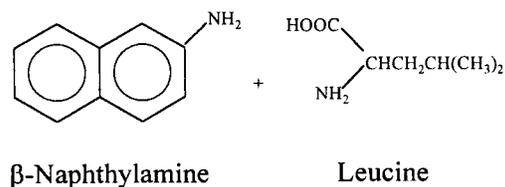
The enzyme amino acid arylamidase [ $\alpha$ -aminoacyl-peptide hydrolase (microsomal) EC 3.4.11.2] catalyzes the release of an N-terminal amino acid from peptides, amides, or arylamides. Because of the presence of such substrates in soils, it is likely that this enzyme is involved in N mineralization. We report on the detection of the activity of this enzyme in soils and describe a precise and accurate method for its assay. This method involves colorimetric determination of the  $\beta$ -naphthylamine produced when soil is incubated with L-leucine  $\beta$ -naphthylamide in 0.1 M THAM [tris(hydroxymethyl)aminomethane] buffer (pH 8.0) at 37°C for 1 h. The  $\beta$ -naphthylamine that is produced is extracted with ethanol and converted into an azo compound by reacting with *p*-dimethylaminocinnamaldehyde, and the absorbance of the color is measured at 540 nm. This enzyme has its optimal activity at pH 8.0 and is inactivated at temperatures above 60°C. Preheating soil samples for 2 h at temperatures ranging from 20 to 120°C before assay showed that this enzyme is stable up to 40°C in field-moist soils and up to 60°C in air-dried samples. The  $K_m$  values of arylamidase activity in seven surface soils ranged from 0.19 to 0.35 mM. The temperature response followed Arrhenius equation over the range from 20 to 50°C. The activation energy values ranged from 30.6 to 49.8 kJ mol<sup>-1</sup> for field-moist soils and from 26.2 to 32.4 kJ mol<sup>-1</sup> for their air-dried counterparts. The means of temperature coefficients ( $Q_{10}$ ) ranged from 1.32 to 1.71 (avg. = 1.44). Several compounds inhibited the activity of this enzyme in soils.

THE ENZYME AMINO ACID ARYLAMIDASE [ $\alpha$ -aminoacyl-peptide hydrolase (microsomal) EC 3.4.11.2] catalyzes the hydrolysis of an N-terminal amino acid from peptides, amides, or arylamides. Arylamidase is widely distributed in the tissues and body fluids of all animals (Hiwada et al., 1980), plants, and microorganisms (Appel, 1974). The chemical nature of N in soils is such that a large proportion (15–25%) of organic N is often released as NH<sub>4</sub><sup>+</sup> by 6 M HCl hydrolysis. The information available suggests that a portion of the released NH<sub>4</sub><sup>+</sup> is derived from amino acid residues in linear amides, and arylamides of soil organic matter (Sowden, 1958). Several papers have been published on linear amidase in soils and on the enzyme involved in hydrolysis of amides and amino acids such as asparagine, aspar-

tic acid, and glutamine (Frankenberger and Tabatabai, 1980, 1991a, 1991b; Senwo and Tabatabai, 1996), but the possibility of the presence of arylamidase in soils has not been explored. The activity of this enzyme in soils deserves investigation because present knowledge indicates that a variety of arylamides are present in soils (Stevenson, 1994). Arylamidase may play an important role as an initial limiting step in mineralization of organic N in soils. Thus, understanding the environmental controls on the activity of this enzyme in soil is important for better understanding the N-cycling process. This enzyme is capable of hydrolyzing the neutral amino acid  $\beta$ -naphthylamides or *p*-nitroanilides according to the following reaction (using the amino acid L-leucine as an example):



L-Leucine  $\beta$ -Naphthylamide



$\beta$ -Naphthylamine

Leucine

The objectives of the present work were (i) to develop a simple and sensitive method for the assay of arylamidase in soils and to ascertain the factors affecting the observed activity, and (ii) to determine the kinetic parameters of the reaction catalyzed by this enzyme. The method developed involves colorimetric determination of the  $\beta$ -naphthylamine produced by arylamidase activity when soil is incubated with 0.1 M THAM buffer (pH 8.0) and L-leucine  $\beta$ -naphthylamide hydrochloride at 37°C for 1 h. The factors studied included pH, substrate

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**Abbreviations:** THAM, [tris(hydroxymethyl)aminomethane].